interference between this patent application and U.S. Patent 5,380,831 to Adang, et al.

Under 37 CFR § 607(2), applicants present as the proposed count the following:

> A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:
>
> analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and
>
> modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence.

As required under 37 CFR § 1.607(3), applicants identify claim 1 of U.S. Patent 5,380,831 as corresponding to the proposed count. Applicants additionally believe that claim 11 also corresponds to the identified count. Applicants also assert that Adang, et al. claims 2 - 10 and 1 - 14 correspond to the proposed count.

As required by 37 CFR § 1.607(4), applicants identify newly added claims 20, 21, and 22 presented herewith as corresponding to the count.

Under 37 CFR § 1.607(5), applicants, in the following discussion, apply the terms of application claims, identified above as corresponding to the count and not previously to the disclosure of the application. Additionally, applicants have

enclosed a claim chart labelled as Exhibit 1 comparing the above-identified application and the proposed count.

The count requires a method of designing a synthetic Bacillus thuringiensis gene to be more highly expressed in plants, comprising the steps of (1) analyzing the coding sequence of a gene derived from a Bacillus thuringiensis which encodes an insecticidal protein toxin and (2) modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended host plant than did said coding sequence.

Applicants disclose step (1) beginning at page 4, line 33 through page 5, line 31 of the specification. In this section of the specification, applicants address the lack of efficient expression of the native B.t. gene in plant cells and describe the problem as originating in the native B.t. coding sequence itself. Applicants comment that the native B.t. gene has a high proportion of A and T nucleotide bases in the coding sequence relative to other bacterial coding sequences that have been found to be more easily expressed in plants. (Fig. 2 contains the nucleotide sequence of the native B.t. gene.)

At page 5, line 32, applicants begin to disclose their solution to the problem of inefficient B.t. transcription. On page 6 beginning with line 11, applicants propose to examine codons with preferred usage in plant cells. Applicants then describe use of GenBank and EMBL public sequence data to obtain a table of preferred plant codon usage. Fig. 1 in the specification is a result of this endeavor. At page 9, line 1,

applicants compare the codon usage for the native B.t. coding sequence with the codon usage frequency of native plant genes and note that there is a striking difference.

Step (2) of the count is disclosed beginning at page 9, line 30 through page 12, line 7. In these pages applicants describe their decision to construct a synthetic B.t. coding region for a chimeric gene composed primarily of codon selected from those codons which are preferentially expressed by plants as determined by the codon usage pattern illustrated in Fig. 1. Applicants decided to first modify the 5' end of the coding sequence. On page 10, beginning at line 3, applicants describe their decision to design an altered codon usage for the first 138 codons of the B.t. gene. The codons for each codon set of the synthetic region were selected to code for the identical amino acids present in the native protein but were selected to be of the particular codon that has the highest frequency of use according to the plant gene codon analysis in Fig. 1. Fig. 2 of the specification describes a sequence comparison of the original coding region for nucleotides 480 - 903 of the PAMVBTS gene (the native sequence) aligned with the synthetic coding region. On page 11, beginning at line 3, applicants describe their strategy of using three sets of oligonucleotides arranged so that the oligonucleotides would be easily annealed and could create an entire synthetic coding region. Fig. 3 describes the use of oligonucleotides KB 72 and KB 73, Fig. 4 describes the use of KB 74 and KB 75, and Fig. 5 describes the use of KB 76 and KB 77 to derive the final altered sequence. The use of all the oligonucleotides and the final

altered sequence is summarized in Fig. 6. This final "codon switch" sequence contained "a greater number of codons preferred by the intended host plant than did said coding sequence."

Under 37 CFR § 1.608(b), applicants have filed evidence, which consists of the Declarations of Michael J. Miller, Kenneth A. Barton, Sandra Cannon, Mari Lynn Hough (Bennett) and Barry Cohen, which demonstrate that applicants are prima facie entitled to a judgment relative to the effective filing date of Adang, et al. Applicants hereby present an explanation stating with particularity the basis upon which the Applicant is prima facie entitled to the judgment:

The Declaration of Michael J. Miller describes the analysis of plant gene codon usage which resulted in the codon usage table which is the present Fig. 1 of the above-identified patent application. Mr. Miller notes an August 24, 1987 notebook entry (page 1 of Exhibit 1) as containing a narrative description of his desire to compile a table of codon usage in plant genes. Mr. Miller also points to the August 24, 1987 notebook pages as describing his intention to obtain sequences of various plant genes from databases. Note that Mr. Miller describes his intention to compare various bacterial genes, including the B.t. toxin gene, to the codon table he will construct. Exhibit 1 pages 8 - 9 of Mr. Miller's Declaration (recorded August 31, 1987) describes codon comparisons and checks the codon usage in the proposed modified B.t. toxin gene. Mr. Miller declares in paragraph 5 of his declaration that an analysis of the coding sequence of a gene derived from a *Bacillus thuringiensis* which

401

encodes an insecticidal protein toxin and a modification of a portion of this coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host was accomplished by at least August 31, 1987.

Dr. Kenneth A. Barton declares that he, using information provided by Michael J. Miller, designed oligonucleotides KB 72 - KB 77 (described in the above-identified application at Figs. 3, 4 and 5 and page 14, beginning with line 11) and assembled the oligonucleotides into plant expression vector PAMVBTS as illustrated in Fig. 6. Dr. Barton notes that page 1, Exhibit 1 (recorded August 26, 1987) describes his desire to alter the coding region of the B.t. toxin gene and provide more efficient codon usage for plant expression. Dr. Barton points out on page 1 that he and Michael J. Miller decided to sequentially replace the gene beginning at the amino terminus using oligonucleotide spanning approximately 50 codons each. Dr. Barton also notes that page 2 of Exhibit 1 contains an analysis of the B.t. toxin gene in a plant for modifying portions of the gene to yield modified sequences containing a greater number of plant-preferred codons (August 26, 1987). Dr. Barton notes that a successful "codon switch" sequence had been created by at least October 20, 1987.

Sandra Cannon declares that she was employed to create and analyze transgenic plants from the "codon switch" constructs. Ms. Cannon declares that most of this work took place between January, 1988 and July, 1988. Ms. Cannon specifically points to

notebook records in June and July of 1988 that show individual plants being tested for efficacy in killing worms.

The Declaration of Sandra Cannon and Mari Lynn (Bennett) Hough are included to corroborate the Declarations of Mr. Miller and Dr. Barton.

Therefore, these Declarations demonstrate that a plant codon usage table had been created and compared to codon usage in a native B.t. toxin gene by at least August 31, 1987, a synthetic B.t. expression vector using preferred codons for expression of the B.t. toxin had been created by at least October 20, 1987, and the engineered sequence had been inserted into plants and the verification of the toxicity of plants to insects had been established by at least July 18, 1988. Applicants assert that at least by October 20, 1987, all the elements of the count had been conceived and reduced to practice by applicants.

All of the dates in the paragraph above predate the September 9, 1988 filing of Serial No. 242,482, which is the first version of the Adang, et al. patent which supports the present claims. Applicants have reviewed the earlier-filed patent applications from which Adang claims priority, (as exemplified European Patent application corresponding to 535,354) and find the earlier specifications are drawn to expression of native B.t. toxin. No subject matter in the parent cases addresses modification of the coding region for expression in plant as disclosed in U.S. Serial No. 242,482. Therefore, Adang parental patent applications 06/535,354 filed September 26, 1983 and 06/848,733 filed April 4, 1986 are believed not to support

the count. Applicants here have attempted to review U.S. Serial No. 848,733, but were informed by U.S. Patent Office officials that this serial number was not available, because it was involved in an interference action. Therefore, applicants have thus made a prima facie case for judgment against the filing date of September 9, 1988, for U.S. Serial No. 242,482.

Accordingly the declaration of an interference between this patent application and U.S. Patent 5,380,831 is respectfully requested.

Applicants have enclosed a petition and fee for three months extension of time. Please charge deposit account number 17-0055 for this fee. No other fees are believed necessary. However, if further fees are necessary, please charge deposit account number 17-0055.

Respectfully submitted,

Nicholas J. Seay, Reg. No. 27386
Attorney for Applicant
QUARLES & BRADY
P.O. Box 2113
Madison, WI 53701-2113

(608) 251-5000

QBMAD1\85513

142

CLAIM CHART

Count	Specification
A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly express in plants, comprising the steps of:	- Page 3, lines 24 - 30 and page 4, lines 10 - 13.
analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and	- Page 4, line 33 - page 5, line 31 describes an analysis of B.t. toxin gene. - Page 6, line 11 - page 9, line 29 describes the construction of a plant codon usage table. - Fig. 1 is the codon usage table. - Fig. 2 is the nucleotide sequence of the native B.t. toxin gene.
modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence.	- Page 9, line 30 - page 12, line 7, in particular page 9, line 30 - page 11, line 16. - Figs. 3, 4, 5, and 6.